Federal Aviation Administration

DOT/FAA/AM-10/19
Office of Aerospace Medicine
Washington, DC 20591

Postmortem Concentrations of Tramadol and *O*-Desmethyltramadol in 11 Aviation Accident Fatalities

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December 2010

Final Report

OK-11-0024-JAH

NOTICE

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www.faa.gov/library/reports/medical/oamtechreports

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-10/19		

4. Title and Subtitle	5. Report Date
Postmortem Concentrations of Tramadol and *O*-Desmethyltramadol in 11 Aviation Accident Fatalities	December 2010
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Lewis RJ,[1] Ritter RM,[1] Johnson RD,[1] Crump RW[2]	

9. Performing Organization Name and Address		10. Work Unit No. (TRAIS)
[1]Civil Aerospace Medical Institute P.O. Box 25082 Oklahoma City, OK 73125	[2]University of Central Oklahoma Edmond, OK 73034	11. Contract or Grant No.

12. Sponsoring Agency Name and Address	13. Type of Report and Period Covered
Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591	14. Sponsoring Agency Code

15. Supplemental Notes

This work was accomplished under the approved task AM-B-10-TOX-204.

16. Abstract

Tramadol is a centrally acting analgesic used to treat moderate-to-severe pain. Side effects of this medication include dizziness, confusion, drowsiness, seizures, and respiratory depression. Any of these side effects could negatively affect a pilot's performance and become a factor in an aviation accident. Due to the severity of aviation accidents, blood samples are often not available, and frequently, only tissue specimens are available for analysis. Therefore, understanding the distribution of a drug throughout all fluids and tissues of the body is important when trying to interpret drug impairment and/or intoxication. Our laboratory has determined the distribution of tramadol and its main active metabolite, *O*-desmethyltramadol, in various postmortem tissues and fluids obtained from 11 fatal aviation accident cases. Whole blood tramadol concentrations obtained from these 11 cases ranged from 81-2720 ng/mL. When available, 10 specimen types were analyzed for each case, including blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, heart, and brain. Distribution, expressed as specimen/blood ratio, for tramadol was 69 ± 74 in urine, 2.58 ± 3.26 in vitreous humor, 4.90 ± 3.32 in liver, 3.43 ± 2.31 in lung, 3.05 ± 1.49 in kidney, 5.15 ± 2.66 in spleen, 1.18 ± 0.85 in muscle, 2.33 ± 1.21 in brain, and 1.89 ± 1.01 in heart. Distribution coefficients obtained had coefficient of variations (CV) ranging from 49-126%. With such large CV's, the distribution coefficients have little use in predicting blood concentrations from the analysis of a tissue specimen. This study indicates that tramadol concentrations undergo significant postmortem changes.

17. Key Words	18. Distribution Statement
Forensic Toxicology, Tramadol, Distribution, GC/MS, Forensic Toxicology	Document is available to the public through the Defense Technical Information Center, Ft. Belvoir, VA 22060; and the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	10	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

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Postmortem Concentrations of Tramadol and *O*-Desmethyltramadol in 11 Aviation Accident Fatalities

INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) is responsible under Department of Transportation Orders 8020.11B and 1100.2C to "conduct toxicological analysis on specimens from … aircraft accident fatalities" and "investigate … general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of … chemical (use)." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Bioaeronautical Sciences Research Laboratory, where toxicological analysis is conducted on various postmortem fluids and tissues. Occasionally, during a toxicological evaluation, potentially impairing compounds are detected in postmortem specimens from aviation accident victims.

Tramadol (2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)cyclohexanol), sold under the brand name Ultram**,** is a centrally acting analgesic prescribed for the treatment of moderate to severe pain.[1] The Food and Drug Administration approved tramadol in 1995 for legal use in the United States. Impairing side effects from the usage of tramadol include dizziness, confusion, light-headedness or fainting spells, drowsiness, seizures and respiratory depression.[2-4] Tramadol undergoes extensive metabolism by *O*- and *N*-demethylation (Figure 1). Due to its higher affinity for the μ-opioid receptor, *O*-desmethyltramadol has 2-4 times the efficacy of tramadol, whereas, *N*-desmethyltramadol has little therapeutic effect.[4]

Postmortem concentrations of tramadol following drug overdose has been reported in the scientific literature.[1,5-11] However, little information concerning postmortem concentrations and distribution of tramadol at therapeutic levels has been reported.[12] Since scientific information concerning the distribution of tramadol, when present at therapeutic concentrations, is very limited, our laboratory set out to determine its distribution in various postmortem tissues and fluids. A search of our internal laboratory database identified 11 aviation fatalities where tramadol was detected in blood, which also had a full complement of biological tissues and fluids available for further analysis. The specimen types included urine, vitreous humor, lung, liver,

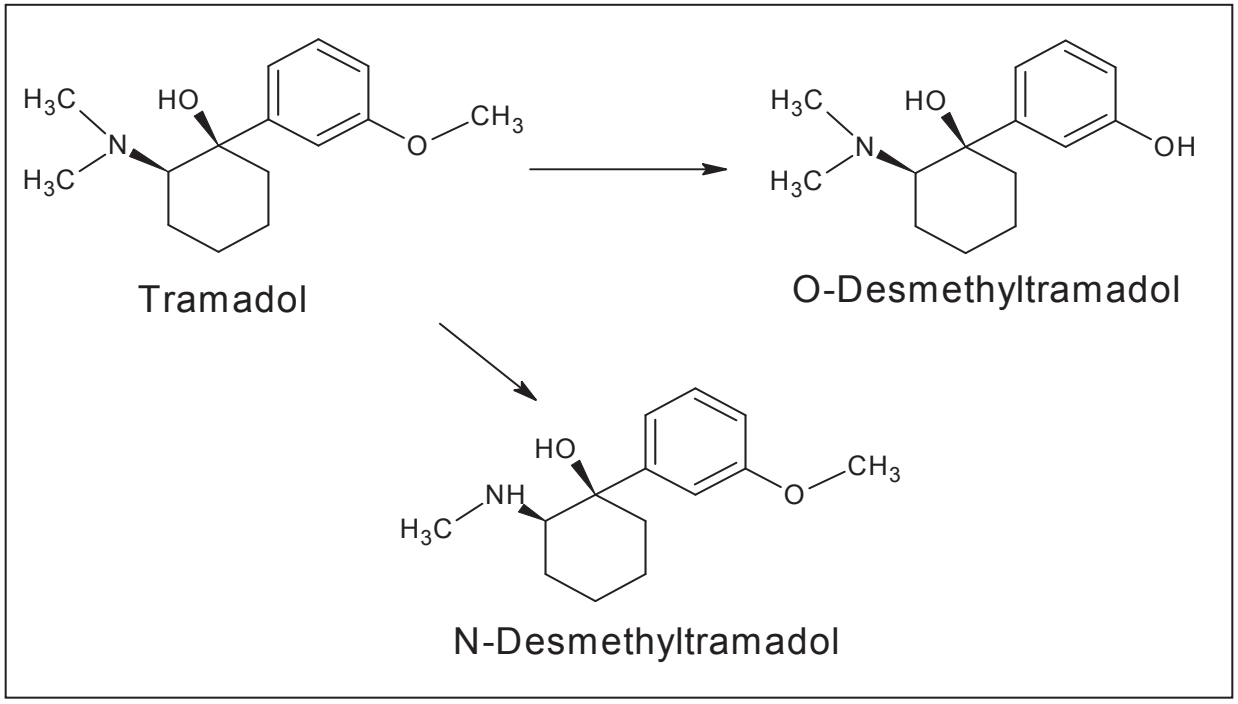

Figure 1. Chemical structures of tramadol and its metabolites.

kidney, spleen, muscle, heart, and brain. This report presents the quantitation and distribution of tramadol in postmortem specimens.

MATERIALS AND METHODS

Chemicals and Reagents

Tramadol and *O*-desmethyltramadol were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) at a concentration of 1.00 mg/mL in methanol. $C_{13}D_3$-tramadol was purchased from Cerilliant at a concentration of 100 µg/mL in methanol. Methanol, acetic acid, potassium phosphate dibasic, sodium fluoride, ammonium hydroxide, and ethyl acetate were purchased from Fisher Scientific (Fisher Scientific, Inc.; Pittsburgh, PA) in the highest possible purity. Double deionized water (DDW), was obtained using an ELGA, PURELAB Ultra water system (ELGA, Inc.; Lowell, MA).

Sample Selection and Storage

A search of the CAMI toxicology database (ToxFLO™, DiscoverSoft Development, LLC; Oklahoma City, OK) identified 11 tramadol-positive fatalities from separate civil aviation accidents that occurred during a period of 3 years (2006-2008). Each of these cases had a majority of the desired biological tissues and fluids available for further analysis (blood, urine, vitreous humor, lung, liver, kidney, spleen, muscle, heart, and brain). In each case, blood was stored at -20°C in tubes containing 1.00% (w/v) sodium fluoride/potassium oxalate prior to analysis. All other specimen types were stored without preservation at -20°C until analysis. Whole-blood tramadol concentrations determined in this study agreed well with those previously determined by our laboratory. In fact, tramadol concentrations found were within 10% of the value originally determined, verifying that no deterioration had occurred under these storage conditions.

Gas Chromatographic/Mass Spectroscopic Conditions

All analyses were performed using a bench top gas chromatograph-mass spectrometer (GC/MS) consisting of a Agilent 6890 series GC connected to an Agilent 5973 quadrupole MS (Agilent; Palo Alto, CA). The GC/MS was operated with a transfer line temperature of 280°C and a source temperature of 250°C. Chromatographic separation was achieved using a FactorFour®, cross-linked, 100% methyl siloxane capillary column (12m x 0.2mm-i.d., 0.33-µm film thickness) obtained from Varian (Varian, Inc.; Santa Clara, CA). The carrier gas utilized was helium, with a flow rate of 1.0 mL/min. One µL was injected into the GC/MS using an Agilent 7683 autosampler. The GC was equipped with a split/splitless injection port operated at 250°C in the splitless mode with the purge time of 0.50 min. The oven temperature profile was 100°C to 290° at 30°C/min, with a final hold time of 0.67 min for a total run time of 7.00 min.

Neat standards of each compound were analyzed individually using the full-scan mode of the GC/MS to select appropriate quantitation and qualifier ions. The MS was run in selected ion monitor (SIM) mode with a dwell time of 20 ms. Ions monitored were *m/z* 263.2, 218.2 and 135.1 for tramadol, *m/z* 249.2, 218.2, and 135.1 for *O*-desmethyltramadol, and *m/z* 267.2, 222.2 and 139.1 for $C_{13}D_3$-tramadol.

Acceptability criteria employed for analyte identification and quantitation were as follows: (1) ion ratios for a given analyte, measured as the peak area of a qualifier ion divided by the peak area of the quantitation ion, were required to be within ± 20% of the average of the ion ratios for each respective calibrator used to construct the calibration curve for that analyte; (2) each ion monitored was required to have a minimum signal-to-noise ratio (S/N) of 10; and (3) the analyte was required to have a retention time within ± 2.00% of the average retention time for each respective calibrator used to construct the calibration curve for that analyte. Analytes not meeting these criteria were reported as either negative or inconclusive.

Calibrator and Control Preparation

Calibration curves for tramadol and *O*-desmethyltramadol were prepared by serial dilution, utilizing bovine whole blood as the diluent. Calibrators were prepared from one set of original stock standard solutions, while controls were prepared in a similar manner as calibrators – but from a second set of unique stock solutions. The calibration curve was prepared at concentrations ranging from 6.25 to 1600 ng/mL. A minimum of six calibrators were used to construct each calibration curve. Controls were prepared at concentrations of 80, 160, 320, and 640 ng/mL and extracted with each batch of unknowns to verify the accuracy of the established calibration curve. Controls were made fresh daily due to breakdown of *O*-desmethyltramadol. An aqueous internal standard solution, $C_{13}D_3$-tramadol, was prepared at a final concentration of 400 ng/mL.

Sample Preparation and Extraction Procedure

All tissue samples were homogenized using an Omni post-mounted homogenizer (Omni International.; Kennesaw, GA). Tissue samples were diluted with a 1.00% NaF solution in a 1:2 (tissue:1% NaF solution) dilution prior to being homogenized. Three mL aliquots of each

calibrator, control, postmortem fluid, and 3.00 g aliquots of each tissue homogenate were transferred into individual 16 x 150-mm screw-top tubes. A 1 mL aliquot of the stock internal standard (400 ng) solution was added to each sample. Six mL of 0.10 M phosphate buffer (pH 6.00) was added to each tube and mixed vigorously. The specimens were then centrifuged at 820 x *g* for 45 min. Following centrifugation, the extracts were transferred to Bond Elute Certify® solid-phase extraction (SPE) columns (Varian, Inc.), which had been pre-conditioned with 2 mL methanol followed by 2 mL of 0.10 M phosphate buffer (pH 6.00). Care was taken to prevent the columns from drying prior to sample addition. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian Cerex® 24-port, positive-pressure extraction manifold with a nitrogen pressure of 3 psi. After each sample passed through its respective column, all SPE columns were washed with 1 mL of 1.0 M acetic acid and dried for 5 min with 25 psi of nitrogen. Once dry, the columns were again washed by adding 6 mL of methanol to each. The columns were again dried with nitrogen at 25 psi for 5 min. The analytes of interest were eluted from the columns with 3 mL of 2% ammonium hydroxide in ethyl acetate and evaporated to dryness in a TurboVap concentration workstation (Caliper Life Sciences; Hopkinton, MA) set at 40°C under a stream of dry nitrogen. Once dried, the residue was reconstituted in 50 µL of ethyl acetate and transferred to GC/MS autosampler vials for analysis.

RESULTS AND DISCUSSION

Analysis of Tramadol

The described procedure, utilizing SPE and GC/MS, proved to be a rapid and sensitive method for the analysis of tramadol and *O*-desmethyltramadol. Analyte peaks were completely resolved, and each provided quantitation ions with unique *m/z*, so no interference was observed. Deuterated tramadol was used as internal standards for this study. This eliminated any concerns over possible matrix effects and allowed for accurate quantitation in specimen types other than whole blood while utilizing a whole blood calibration curve. No analyte suffered interference from endogenous/exogenous matrix components.

The linear dynamic range (LDR) and limit of quantitation (LOQ) for both tramadol and *O*-desmethyltramadol were determined, using bovine whole blood as the matrix. The LDR for each analyte was determined to be 6.25-1600 ng/mL. The calibration curves for tramadol and *O*-desmethyltramadol had correlation coefficients of 0.998 and 0.997, respectively. The LOQ, defined as the lowest detectable analyte concentration that meets all identification criteria discussed in the method section, in addition to being within 20% of its target concentration, for both tramadol and *O*-desmethyltramadol was determined to be 6.25 ng/mL. The LOD, defined as the lowest detectable analyte concentration that meets all identification criteria discussed in the Method section, was determined to be 1.56 ng/mL for tramadol and 3.13 ng/mL for *O*-desmethyltramadol.

Carryover was not found to be a problem on the GC/MS; however, it was initially investigated and subsequently monitored by the use of ethyl acetate blank injections. The injection of an ethyl acetate blank following the 1600 ng/mL calibrator showed no carryover contamination. Subsequently, two ethyl acetate blanks were utilized between each postmortem specimen throughout the sample sequence to verify that no carryover from sample to sample had occurred.

Postmortem Concentrations of Tramadol and *O*-Desmethyltramadol

Tramadol blood concentrations found in these 11 postmortem cases ranged from 0.081 to 2.72 µg/mL. Therapeutic blood concentrations range from 0.150 to 0.800 µg/mL.[5] Toxic levels of tramadol have been reported at concentrations as low as 1.40 µg/mL.[13] Lethal levels of tramadol have been reported at concentrations as low as 2.00 µg/mL.[13] Blood concentrations observed in this study ranged from slightly below therapeutic to toxic levels. However, a previous study found that tramadol concentrations in impaired drivers overlapped with concentrations found in tramadol overdoses.[14] Additionally, the site from which the blood was collected at autopsy is unknown for each of these cases. Thus, due to postmortem redistribution, these blood concentrations may not be representative of the levels observed prior to death. The concentration of these compounds in each postmortem specimen analyzed from these 11 cases can be seen in Tables 1 and 2.

The following mean concentrations (µg/mL, µg/g) of tramadol were found: 0.771 in blood (0.081-2.72, n=11), 29.72 in urine (0.461-152, n=10), 1.01 in vitreous humor (0.092-4.45, n=6), 2.55 in liver (0.303-7.74, n=11), 2.23 in lung (0.075-6.58, n=10) 1.45 in kidney (0.221-3.63, n=11), 2.29 in spleen (0.286-6.69, n=11), 0.583 in muscle (0.077-2.15, n=11), 1.10 in brain (0.117-3.08, n=11), and 0.088 in heart (0.084-2.48, n=11). The following mean concentrations (µg/mL, µg/g) of *O*-desmethyltramadol were found: 0.043 in blood (0.017-0.085, n=5), 4.78 in urine (0.670-9.65, n=9), 0.479 in liver (0.142-1.23, n=7), 0.358 in lung (0.032-1.26, n=7), 0.279 in kidney (0.055-0.914, n=8), 0.298 in spleen (0.062-0.871, n=8), 0.136 in muscle (0.071-0.174, n=3), 0.151 in brain

Table 1. Tramadol concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart
1	0.151	9.59	0.381	1.46	1.11	0.690	1.07	0.259	0.462	0.397
2	0.636	77.17	—	3.17	4.54	2.00	3.96	0.664	1.39	1.80
3	0.606	152.03	1.04	7.74	—	3.41	6.69	2.15	2.87	1.67
4	2.72	15.87	—	4.80	6.58	3.63	3.52	1.33	3.08	2.48
5	0.222	7.36	—	1.36	0.950	1.17	1.44	0.306	0.806	0.697
6	2.50	23.51	0.084	6.61	4.34	2.60	4.50	0.927	1.85	1.31
7	0.260	6.90	—	0.880	3.37	0.851	1.25	0.244	0.420	0.502
8	0.462	3.40	4.45	1.13	0.798	0.942	1.493	0.284	0.597	0.398
9	0.093	1.05	0.038	0.157	0.075	0.146	0.286	0.054	0.117	0.084
10	0.091	0.461	—	0.429	0.353	0.273	0.602	0.120	0.313	0.301
11	0.081	—	0.092	0.303	0.115	0.221	0.405	0.077	0.206	0.086

* All concentrations shown in units of μg/mL or μg/g
— Specimen type not available for analysis

Table 2. O-Desmethyltramadol concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart
1	0.017	1.730	neg	0.142	0.063	0.090	0.062	neg	0.045	0.039
2	neg	9.65	—	1.23	1.26	0.914	0.871	0.174	0.268	0.347
3	0.031	9.53	neg	0.492	-	0.183	0.275	0.071	0.157	neg
4	neg	0.744	—	neg	0.130	0.102	neg	neg	0.053	neg
5	0.056	7.16	—	0.228	0.093	0.189	0.191	neg	0.194	0.146
6	neg	5.45	neg	0.932	0.540	0.601	0.619	0.164	0.300	neg
7	neg	6.23	—	0.149	0.389	neg	0.148	neg	neg	neg
8	0.085	1.87	neg	0.178	neg	neg	neg	neg	neg	neg
9	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg
10	neg	0.670	—	neg	neg	0.094	0.146	neg	neg	neg
11	0.028	—	neg	neg	0.032	0.055	0.070	neg	0.038	neg

* All concentrations shown in units of μg/mL or μg/g
— Specimen type not available for analysis

(0.038-0.300, n=7), and 0.177 in heart (0.039-0.347, n=3), and each vitreous humor specimen was negative.

The distribution coefficients for tramadol, expressed as specimen concentration/blood concentration, were found to be: 69 ± 74 urine, 2.58 ± 3.26 vitreous humor, 4.90 ± 3.32 liver, 3.43 ± 2.31 lung, 3.05 ± 1.49 kidney, 5.15 ± 2.66 spleen, 1.18 ± 0.85 muscle, 2.33 ± 1.21 brain, and 1.89 ± 1.01 heart. The distribution coefficients for *O*-desmethyltramadol, expressed as specimen concentration/blood concentration, were 140 ± 121 urine, 7.60 ± 6.10 liver, 6.51 ± 1.36 lung, 4.13 ± 1.80 kidney, 4.61 ± 2.89 spleen, 3.13 ± 1.55 brain, and 2.45 ± 0.22 heart. No distribution coefficient for *O*-desmethyltramadol was determined in either vitreous humor or muscle due to an insufficient number of replicates for these specimen types. A detailed listing of the distribution coeficients are shown in Tables 3 and 4.

Tramadol distribution coefficients obtained in this study had coefficient of variation (CV) values that ranged between 49 and 126%. The same tissue samples produced CV values for *O*-desmethyltramadol that ranged from 9 to 80%. The large CV values associated with the distribution coefficients obtained suggest a high degree of inter-individual variation in the distribution of these compounds. This inconsistency could result from numerous factors, sush as differing blood collection sites at autopsy, postmortem interval, postmortem redistribution, and contamination. The blood collection site and postmortem interval for these cases are unknown. However, in most of our cases in which the collection site is noted, the blood typically originates from the chest cavity. Alkaline compounds readily undergo postmortem redistribution in the interval between death and specimen collection. This redistribution could account for some of the larger CV values obtained. Additionally, with a relatively small volume of distribution (3 L/kg),[13] one would expect tramadol concentrations obtained from tissue specimens to be similar to those found in whole blood. This was not consistent with our findings, which suggests that postmortem changes in drug concentrations had occurred.

Drug concentrations determined from a blood specimen can aid in determining impairment and/or cause of death. However, due to the destructive nature of aviation accidents, our laboratory receives blood in only approximately 70% of the cases examined. If a distribution coefficient with a CV < 20% is determined, it may be possible, with caution, to use a tissue or fluid distribution coefficient to roughly estimate a blood concentration in cases where blood is not available for analysis. However, the results obtained from our limited number of cases show that tramadol blood concentrations cannot be estimated, even crudely, from other tissue/fluid concentrations.

Table 3. Postmortem tissue distribution coefficients for Tramadol.

	Urine/ Blood	VH*/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood
n	10	6	11	10	11	11	11	11	11
Mean	69.43	2.58	4.90	3.42	3.05	5.15	1.18	2.33	1.89
s.d.	74.38	3.26	3.32	2.31	1.49	2.66	0.845	1.21	1.01
CV	107.0	126.0	67.6	67.4	49.0	51.7	72.6	51.9	53.4

* vitreous humor

Table 4. Postmortem tissue distribution coefficients for O-Desmethyltramadol.

	Urine/ Blood	VH*/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood
n	4	—	4	3	4	4	—	4	2
Mean	139.76	—	7.60	6.51	4.13	4.61	—	3.13	2.45
s.d.	120.5	—	6.10	1.36	1.80	2.89	—	1.55	0.221
CV	86.2	—	80.2	20.8	43.5	62.2	—	49.5	9.02

* vitreous humor

CONCLUSION

Our laboratory identifies numerous tramadol-positive cases each year. The undesirable side effects associated with this medication is of concern in the aviation community. With this in mind, a method for the identification and quantitation of tramadol and its active metabolite, *O*-desmethyltramadol, has been developed that is rapid, reliable, and sensitive. By utilizing SPE, a clean extract was achieved that required minimal time and solvent. A total of 103 tissue and fluid samples from 11 deceased individuals were measured to determine tramadol and *O*-demethyltramadol concentrations. Tramadol concentrations ranged from slightly below therapeutic to levels that have been shown to be lethal in some cases. The results obtained from these cases suggest that tramadol is readily absorbed by all tissues and fluids in the body. The CV values obtained for the calculated distribution coefficients were large; therefore, the tramadol distribution coefficients obtained should not be used to estimate drug concentrations in whole blood from available tissue concentrations.

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